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Federal Water Pollution Control Administration
Division of Water Quality Research
Analytical Quality Control Laboratory
Cincinnati, Ohio

CLEAN WATER

METHOD SELECTION STUDY 1:

A COMPARISON OF THREE MODIFICATIONS OF THE SINGLE REAGENT METHOD FOR SOLUBLE ORTHOPHOSPHATE

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METHOD SELECTION STUDY 1

A Comparison of Three Modifications of the Single Reagent Method for Soluble Orthophosphate

Conducted for
The Committee on Method Validation and Quality Control (COMVAQC)

by

Analytical Quality Control Laboratory

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John A. Winter* and Robert L. Booth**

Summary

Recoveries of soluble orthophosphate added to distilled and natural water samples at the 0.2 mg P/1 level were compared using modifications of the mixed reagent method suggested by Edwards, Molof, and Schneeman (1), and Murphy and Riley (2). The method of Murphy and Riley was shown to have the best precision and accuracy and the least bias. It is selected as the FWPCA Interim Official Method.

*Chief, Methods and Performance Evaluation, Analytical Quality Control Laboratory, Division of Water Quality Research, FWPCA
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METHOD SELECTION STUDY 1

A Comparison of Three Modifications of the Single Reagent Method for Soluble Orthophosphate

Introduction

After a review of methods of analysis for soluble orthophosphate, the Nutrient Analyses Subcommitee of the Committee on Method Validation and Quality Control (COMVAQC) chose three modifications of the mixed reagent method for evaluation. These were presented at the second meeting of COMVAQC, October 17-18, 1967, and during this meeting nine laboratories of the Federal Water Pollution Control Administration agreed to cooperate in a round-robin test for selection of the FWPCA Interim Official Method. The study was conducted by the Analytical Quality Control (AQC) Laboratory working with Mr. Robert L. Booth, chairman of the phosphorus task group, COMVAQC.

The cooperators were:

FWPCA Laboratory	Supervisor
Pacific Northwest Water Laboratory Corvallis, Oreg.	Daniel F. Krawczyk
Hudson-Delaware Basins Office Edison, N.J.	Richard T. Dewling
Chicago Program Office Chicago, Ill.	Clifford Risley, Jr.
California/Nevada Basins Project Alameda, Calif.	Robert E. White
Chesapeake Field Station Annapolis, Md.	Donald W. Lear
Technical Advisory & Investigations Branch Cincinnati, O.	Carl R. Hirth

Columbia River Basin Project
Portland, Oreg.
W. Allan Moore

Advanced Waste Treatment
Cincinnati Water Research Laboratory
Cincinnati, O.
Robert T. Williams

Analytical Quality Control Laboratory
Cincinnati, O.
Robert L. Booth

Study Design

The laboratories analyzed a distilled water containing an unknown phosphorus addition furnished by the Analytical Quality Control Laboratory, a natural water of their choice, and the same natural water after addition of the level of phosphorus present in the distilled water unknown:

Standard Solution of Phosphorus (Unknown to Analyst)

Addition of Phosphorus 0.228 mg/l	Addition of Phosphorus 0.228 mg/l	
Natural Water + Phosphorus	Distilled Water + Phosphorus	Natural Water
Analyses by Methods A, B, & C	Analyses by Methods A, B, & C	Analyses by Methods A, B, & C
Result 1	Result 2	Result 3

Three modifications of the mixed reagent method of analysis for soluble orthophosphate were tested:

Method	Reference
A	Edwards, G., Molof, A., and Schneeman, R., "Determination of Orthophosphate in Fresh and Saline Waters," J.AWWA, Vol. 57, 1958, 917.

B Murphy, J. and Riley, J., " A Modified Single Solution Method for the Determination of Phosphorus in Natural Waters," Anal. Chim. Acta, Vol. 27, 1962, 31.

C Modification from: Edwards, G., Molof, A., and Schneeman, R., "Determination of Orthophosphate in Fresh and Saline Waters," J. AWWA, Vol. 57, 1958, 917.

The methods differ in reagent concentrations, in ratio of mixed reagent to sample volume, and in the use of a solvent for color development as follows:

Reagent	Method A	Method B	Method C
Sulfuric Acid	50 ml of 8 N	50 ml of 5 N	50 ml of 5 N
Potassium Antimonyl Tartrate	5 ml of 4.3888 g/200 ml H_2O	5 ml of 0.5486 g/200 ml H_2O	5 ml of 2.1944 g/200 ml H_2O
Mixed Reagent Vol. / Sample Vol.	$\frac{2.5}{50}$	$\frac{8}{50}$	$\frac{5}{25}$
Solvent	2.5 ml ethanol	No solvent	3 ml acetone

Each laboratory was asked to analyze all samples (distilled water + phosphorus, natural water, and natural water + phosphorus) in triplicate and to return all results without averaging. The analytical procedures for each method were provided with instructions for exact sample preparation and a preprinted report form (see Appendix 1).

After all of the data were returned to the AQC Laboratory, they were compared by sample, by analytical method, and by laboratory. A Chi-square test was applied to the data for each method for distilled and natural water samples to determine if the data were normally distributed. Data were then treated statistically according to Natrella (3), Dixon and

Massey (4), and ASTM Manual STP 15-C (5).

Distilled Water Samples

For any one method, Result 2 (Distilled Water + Phosphorus) indicated the accuracy and precision of the method in the hands of the laboratory analyst. Individual results were averaged to obtain one value per method. Mean and range values, estimated standard deviation, coefficient of variation, and 95% confidence limits were calculated. Precision of the three methods was expressed as the coefficient of variation. Accuracy was expressed as the percent deviation from the amount of phosphorus added.

The inability of a laboratory to recover phosphorus in distilled water by any of the methods indicated a problem in technique in the laboratory and allowed separation of these values. This assured the use of data only from those laboratories correctly applying the three methods.

Natural Water Samples

The data on natural water samples were checked for outliers, and for normal distribution, then evaluated statistically for mean values, estimated standard deviation, range, bias and 95% C.L. values. Precision was expressed as the coefficient of variation. Accuracy was expressed as the percent deviation from the amount of phosphorus added. Result 1 minus Result 3 gauged the ability of a method to recover phosphorus from natural waters and in the presence of possible interferences. A major difference between Result 2 and Result 1 minus Result 3 would indicate interference or a lack of ruggedness in the method.

Results

Introduction

Most of the laboratories measured phosphorus recovery in more than one water sample, thereby increasing the number and scope of natural waters examined. The samples included fresh water, estuarine water, sea water, and sewage effluent. Although nine laboratories began the study, failure of one laboratory to recover the spike led to rejection of all of this laboratory's data. Data from the eight remaining laboratories are summarized in Table 1 and the detailed results given in Appendix 2.

Rejection of Outliers - Some recoveries of the phosphorus added to natural samples were unduly affected by positive or negative interferences in the sample. To assure that extreme values did not unfairly influence the method evaluations, the Dixon Criterion (4) test for outliers was applied to the average values from each laboratory for all natural samples. At the 95% significance level, the low and high values noted with (†) were rejected as outliers. They are presented for comparison in Table 1, but were not used in the statistical treatment of data.

Chi-Square Test - A Chi-square test applied to all of the data categorized by method and type of sample showed normal distribution for the data in each group:

Distilled Water Samples	X^2 Value	Critical Value	Distribution
Method A	1.63	11.07	Normal
Method B	1.63	11.07	Normal
Method C	1.32	11.07	Normal
Natural Water Samples			
Method A	3.95	11.07	Normal
Method B	3.96	11.07	Normal
Method C	3.96	11.07	Normal

Method Comparison - Table 2 shows the statistical comparison of the three methods on analysis of the distilled water sample. Method B shows the smallest range, estimated standard deviation and 95% confidence interval. The standard precision measure, coefficient of variation, was 3.03% for Method B, compared to 3.87% and 5.19% for Methods A and C. The accuracy measure, percent deviation from the amount added was 2.38% for Method B or only half that for Methods A, (4.01%) and C (4.38%).

Salt - vs. Fresh-Water Samples - Since the recoveries of phosphorus in natural waters may be dependent on the characteristics of the sample, the natural water data were plotted on probability paper to detect the effect of salt and fresh waters on the recovery by each of the three methods. In Figures 1, 2, and 3, the phosphorus recovery values (by difference) are plotted for Methods A, B, and C in salt and fresh water. Although there is a discontinuity in the plot of the fresh water data from Method B, a X^2 test indicated normality. An inspection of the values in Table 1 shows that all values for Method B were less or slightly more than the added level. This resulted in the apparent step-wise character of data with extremely good precision and accuracy. As with the distilled water data, Method B shows the least slope (greatest precision).

Iron Interference - There was an interference with phosphorus recovery with two natural water samples. One sample from an acid mine drainage stream containing 950 mg/l of dissolved iron at a pH of 2.8 showed erratic high and low values with floc formation (Laboratory 7). The second sample was from a harbor highly polluted with industrial wastes and reported to contain about 2.5 mg Fe/liter (Laboratory 4).

Murphy and Riley (2) reported that their method was not affected by 50 mg/l of ferric iron. Tests completed on Method B in the Methods and Performance Evaluation, AQC Laboratory, determined the point for iron interference (presumably between 50 and 950 mg/l) was > 100 mg/l.

Sewage Effluent - Since only one sewage effluent sample was analyzed, no conclusions can be made about this waste as an interference. Laboratory 3 showed good recovery from sewage with Method B, but bias with Methods A and C for the distilled and natural water samples.

Summary Plot of Phosphorus Recoveries by Methods A, B, and C

With good response of each method in salt and fresh water samples, data from both these sample types were combined for each of the three methods and plotted again on probability paper. These data are shown in Figures 4 and 5.

The three methods show comparable ability to measure phosphorus in distilled water and natural waters. In both graphs, however, Methods B, A, and C show an increasing slope of lines; that is, a descending order of precision. A statistical summary of data from the recoveries of phosphorus added to natural waters is given in Table 3. As with the distilled water samples, Method B shows the smallest bias, the narrowest range of values at 95% Confidence Limit, and the best precision and accuracy, 1.75% and 1.39%, of the three methods.

Summary

Comparison of phosphorus recoveries from natural water samples by the three mixed reagent method modifications shows good precision (2.17%, 1.75%, and 2.71% coefficient of variation) and good accuracy (2.08%, 1.39%, and 3.10% deviation from the amount added) for Methods A, B, and C, respectively. Method B was the best of the three, with 1.75% precision and 1.43% accuracy.

Sea water did not interfere in the methods. Iron was a negative interference in all three methods in an acid mine drainage sample containing 950 mg/l of ferrous and ferric iron. It did not interfere with phosphorus recovery from samples containing up to 100 mg/l of ferrous or ferric iron.

Based on these data, Method B is selected as best of the available methods for use in FWPCA.

References

1. Edwards, G., Molof, A., and Schneeman, R., "Determination of Orthophosphate in Fresh and Saline Waters," J. AWWA, Vol. 57, 1958, 917.

2. Murphy, J. and Riley, J., "A Modified Single Solution Method for the Determination of Phosphorus in Natural Waters," Anal. Chim. Acta, Vol. 27, 1962, 31.

3. Natrella, M. G., Experimental Statistics , NBS Handbook 91, August, 1963.

4. Dixon, W. J. and Massey, F. J. Jr., Introduction to Statistical Analysis , 2nd ed., 1957.

5. ASTM Manual on Quality Control of Materials E-11, published by ASTM, January, 1951.

Table 1

Comparison of Recoveries of 0.228 mg/l Phosphorus from Distilled and Natural Waters by the Methods A, B, and C. Average Values of Soluble Orthophosphate, mg P/l

	Distilled Water Samples			Natural Water Samples (by difference)		
Lab No.	Method A	Method B	Method C	Method A	Method B	Method C
1	.249	.242	.255	.233	.227	†.256
2	.224	.224	.228	.221	.227	.215
3	.243	.229	.211	†.261	.232	.222
4	.225	.235	.220	†.183	†.104	†.092
5	.238	.232	.225	.240	†.210	.212
5	.238*	.232*	.225*	.235	.235	.216
6	.220	.221	.230	.234	.231	.230
7	.237	.236	.242	.230	.230	.222
7	.237*	.236*	.242*	.232	.233	.229
7	.237*	.236*	.242*	.227	.226	.226
8	.225	.225	.233	.225	.225	.227
8	.232	.233	.236	.223	.223	.214
8	.227	.230	.231	.228	.226	.223
Range of Recovery	.029	.021	.044	.019	.012	.018

*Repetition of values for comparison with other water samples. Not used in statistical evaluation.

†Values given for comparison only. These are outliers at 95% significance level, and are not used in statistical evaluation.

Table 2

Data Evaluation from Recoveries of 0.228 mg/l Soluble Orthophosphate, mg P/l from Distilled Water by Methods A, B, and C.

Measure	Method A	Method B	Method C
Number of Laboratories	8	8	8
Number of Samples	10	10	10
Mean value, mg/l	0.232	0.231	0.231
Bias	+.004	+.003	+.003
Range, mg	0.045	0.039	0.068
Estimated Standard Deviation (s), mg/l	±0.009	±0.007	±0.012
95% Confidence Interval, mg/l	0.226-0.238	0.227-0.235	0.224-0.238
Precision in Percent (Coefficient of Variation)	3.87	3.03	5.19
Accuracy in Percent (Deviation from amount added)	4.01	2.38	4.38

Table 3

Data Evaluation from Recoveries of 0.228 mg/1 Soluble Orthophosphate, mg P/1 from Natural Waters by Methods A, B, and C. (Result 1 minus Result 3)

Measure	Method A	Method B	Method C
Number of Laboratories	8	8	8
Number of Samples	11	11	11
Mean Value, mg/1	0.230	0.229	0.221
Bias, mg/1	+.002	+.001	-.007
Range, mg/1	.019	.012	.018
Estimated Standard Deviation (s), mg/1	±.005	±.004	±.006
95% Confidence Interval, mg/1	.233-.227	.227-.231	.217-.225
Precision of Percent (Coefficient of Variation)	2.17	1.75	2.71
Accuracy in Percent (Deviation from amount added)	2.08	1.39	3.10

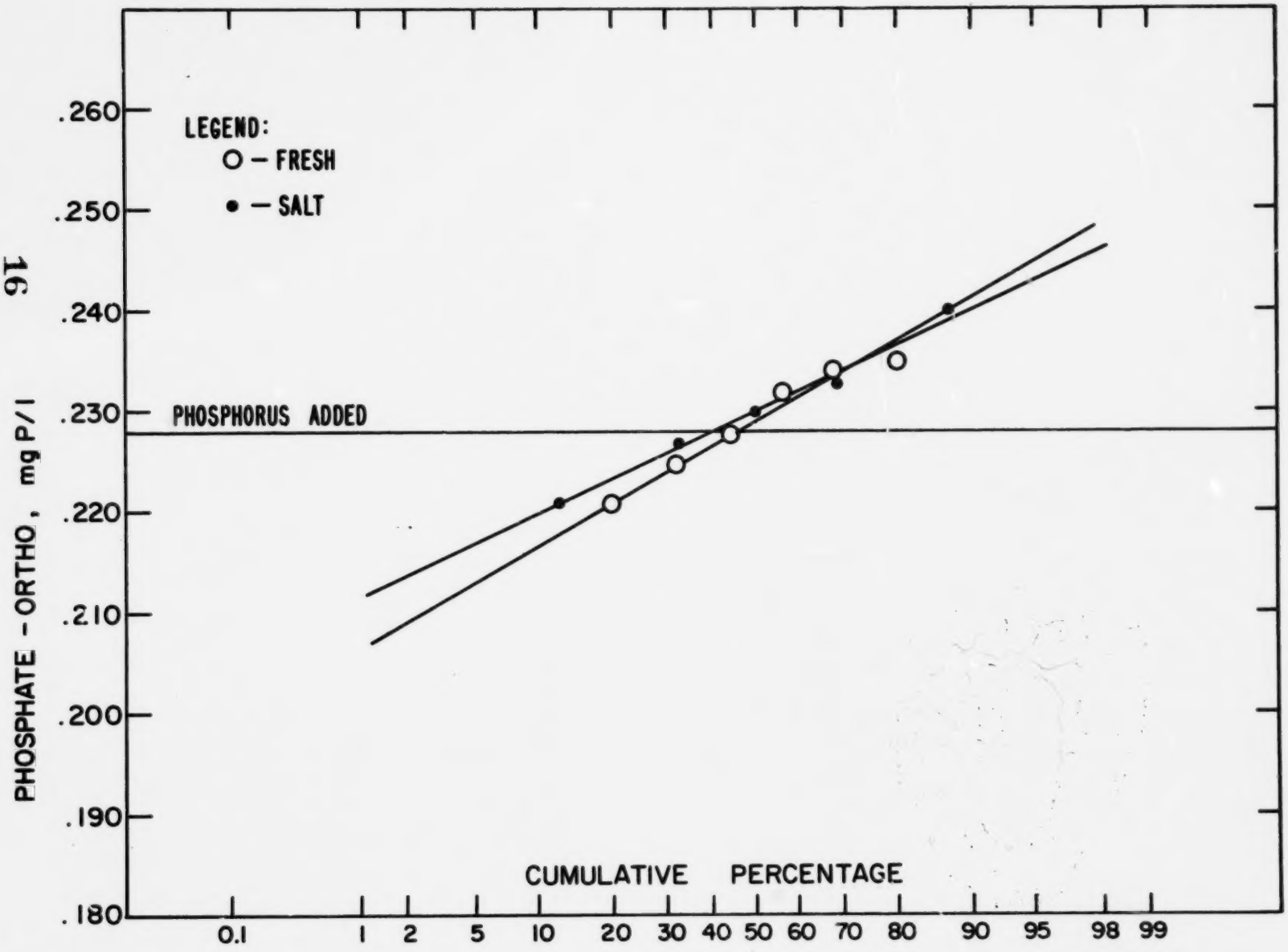

FIGURE 1. RECOVERY OF PHOSPHORUS, FRESH & SALT WATER SAMPLES, METHOD A.

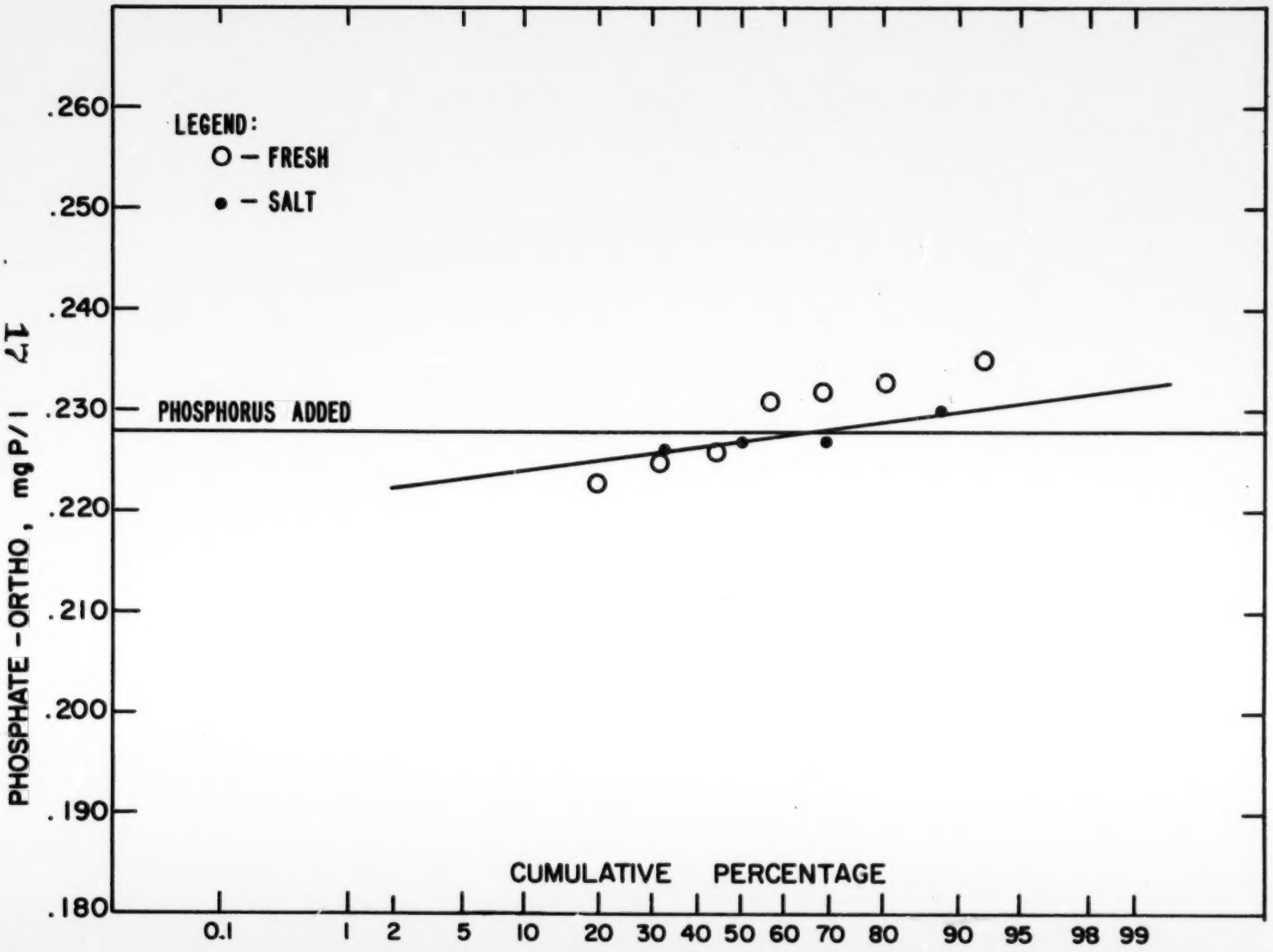

FIGURE 2. RECOVERY OF PHOSPHORUS, FRESH & SALT WATER SAMPLES, METHOD B.

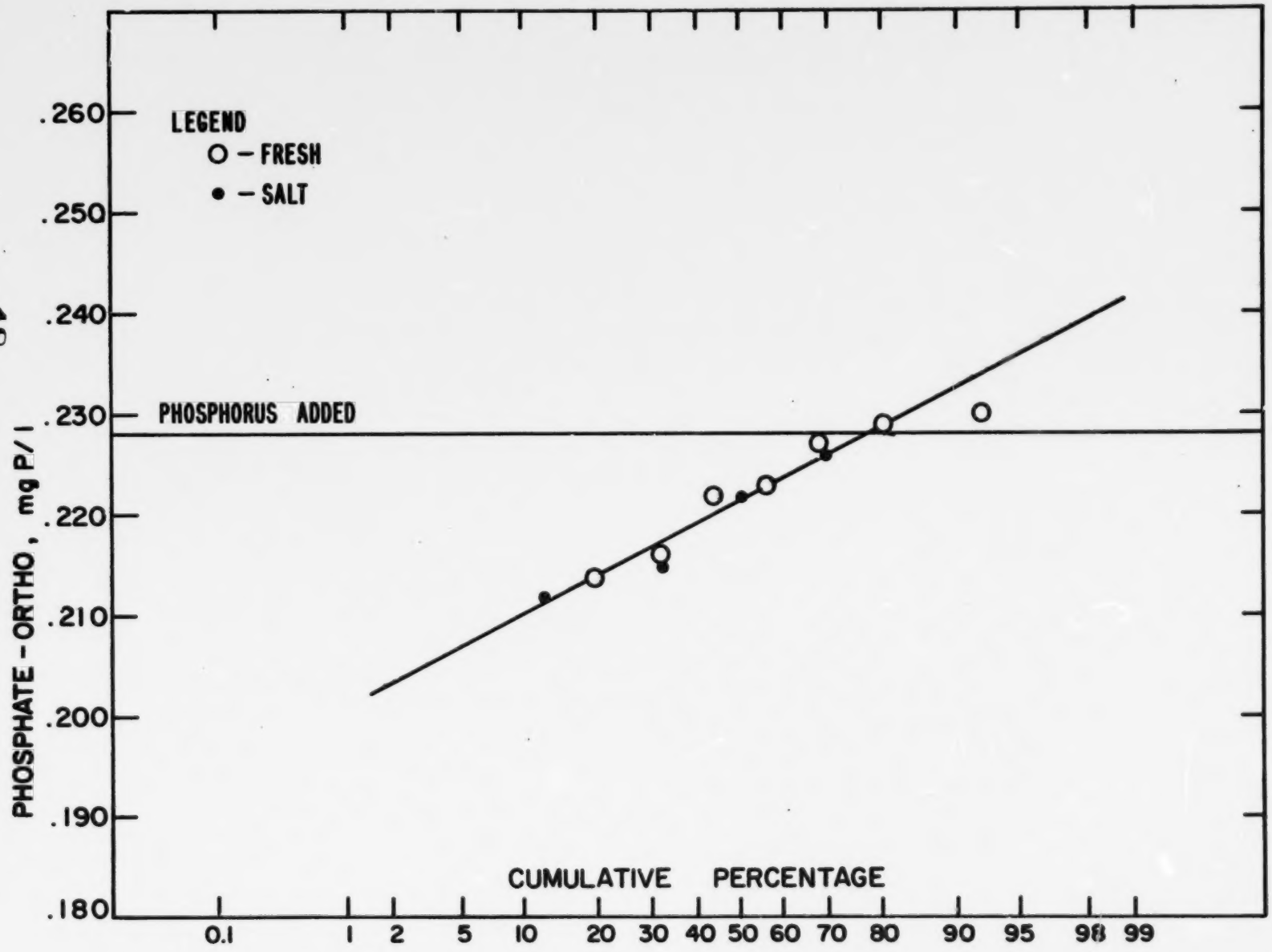

FIGURE 3. RECOVERY OF PHOSPHORUS, FRESH & SALT WATER SAMPLES, METHOD C.

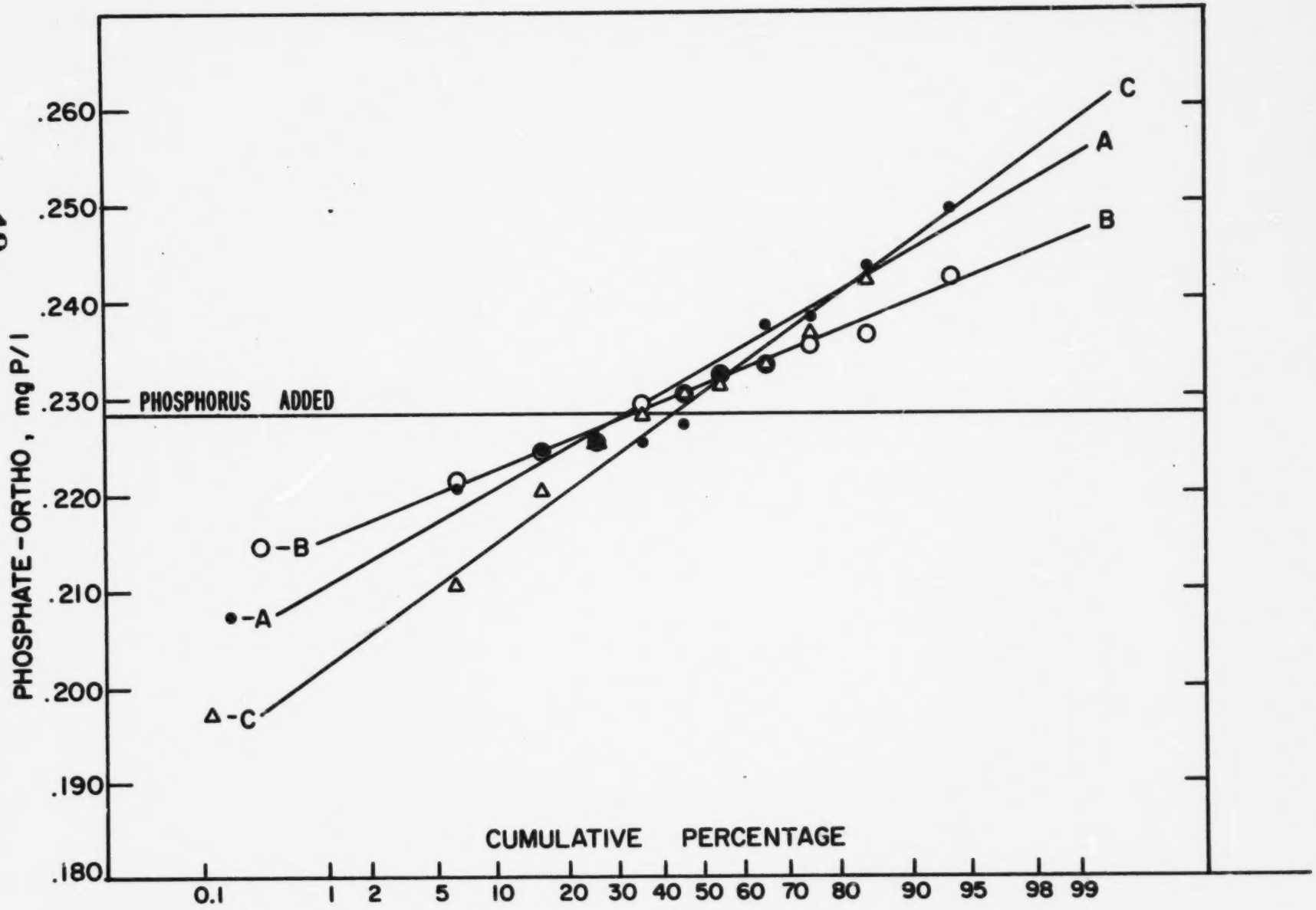

FIGURE 4. PROBABILITY PLOT OF ALL DISTILLED WATER SAMPLES ANALYZED BY METHOD A, B & C.

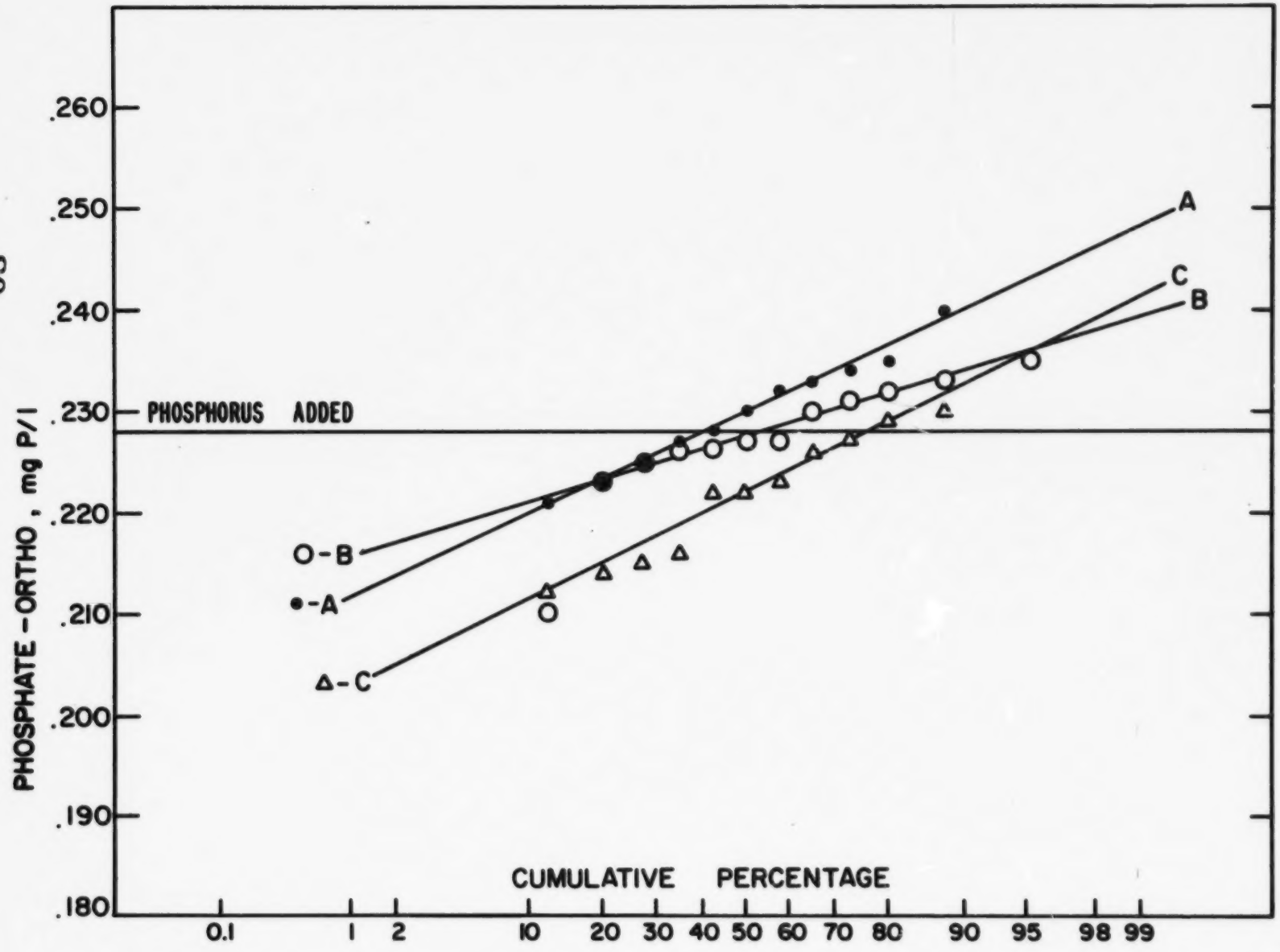

FIGURE 5. PROBABILITY PLOT OF ALL NATURAL WATER SAMPLES ANALYZED BY METHODS A, B & C.

APPENDIX 1

1. Test Instructions: Cover Memorandum
   Instruction Sheet and Report Form.
2. Soluble Orthophosphate Method A
3. Soluble Orthophosphate Method B
4. Soluble Orthophosphate Method C

FEDERAL WATER POLLUTION CONTROL ADMINISTRATION
Division of Water Quality Research
1014 Broadway
Cincinnati, Ohio 45202

OPTIONAL FORM NO. 10
MAY 1962 EDITION
GSA FPMR (41 CFR) 101-11.6

UNITED STATES GOVERNMENT

Memorandum

TO : See Below

DATE: November 6, 1967

FROM : John A. Winter, Chief
Methods & Performance Evaluation
Analytical Quality Control Laboratory, FWPCA

SUBJECT: Special Method Comparisons for Phosphorus Analyses

The round-robin study discussed during the second meeting of COMVAQC in October has been planned for comparison of three methods. Enclosed are copies of the methods, an instruction sheet, and a report sheet. The phosphorus solution for spiking was sent separately and should arrive in a day or two. Water samples should be analyzed as soon as possible after spiking of the samples. The triplicate results on each sample and by each method should be reported directly to me within two weeks.

If there are any questions don't hesitate to contact me at are code 513, 684-2983.

John A. Winter

Attachments

TO: D. Krawczyk, Pacific Northwest Water Laboratory
Corvallis, Oreg.
W. A. Moore, Columbia River Basin Project
Portland, Oreg.
R. Dewling, Hudson-Delaware Basins Office
Edison, N.J.
L. Scarce, Chicago Program Office
Chicago, Ill.
R. White, Central Pacific Project
Alameda, Calif.
D. Lear, Chesapeake Field Station
Annapolis, Md.
R. Williams, Cincinnati Water Research Laboratory
Cincinnati, O.
C. Hirth, Technical Advisory & Investigations Branch
Cincinnati, O.
M. Gales, Analytical Quality Control Laboratory
Cincinnati, O.

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November 6, 1967

INSTRUCTION SHEET FOR SAMPLE PREPARATION
AND ANALYSES FOR SOLUBLE ORTHOPHOSPHATE

1. Prepare reagents for all three methods.

2. Obtain a typical water sample for your area and prepare samples for analysis as described below:

3. Prepare Sample 1 by adding 10.0 ml of unknown (use volumetric pipette) to 990 ml of the collected sample. Mix thoroughly.

   Prepare Sample 2 by adding 10.0 ml of unknown (use volumetric pipette) to 990 ml of distilled water. Mix thoroughly.

   Sample 3 is the unspiked water sample.

4. Filter each sample. Use a glass-fiber prefilter, if necessary, with final filtration through a phosphorus-free membrane filter of 0.45 micron pore size.

5. Take three 50.0 ml aliquots for Method A, three 50.0 ml aliquots for Method B, and 25.0 ml aliquots for Method C from each sample for the determinations of soluble orthophosphate.

6. Report all results as P, mg/l on the attached data sheet. Report only individual results - do not average.

NOTE: For river waters which contain greater than 0.5 mg P/liter, dilution is necessary as the enclosed methods are applicable in the 0 - 0.6 mg P/liter range. Make appropriate dilutions before addition of the unknown.

AQC-COMVAQC
ROUND-ROBIN STUDY

Soluble Orthophosphate, mg P/L

	METHOD A Fuller (Molof)	METHOD B Lear (Murphy & Riley)	METHOD C Lefcourt (Modified Molof)
Sample 1	1)_____	1)_____	1)_____
	2)_____	2)_____	2)_____
	3)_____	3)_____	3)_____
Sample 2	1)_____	1)_____	1)_____
	2)_____	2)_____	2)_____
	3)_____	3)_____	3)_____
Sample 3	1)_____	1)_____	1)_____
	2)_____	2)_____	2)_____
	3)_____	3)_____	3)_____

Laboratory_____ Date of Sample Preparation_____

Analyst_____ Date of Analyses_____

Sample Type_____

Comments or Remarks:_____

Soluble Orthophosphate

Method A*

Recommended by Mr. Fred Fuller, Chicago Program Office
Great Lakes Region, FWPCA

1. Apparatus

1.1 Acid-washed glassware: All glassware used in the determination should be washed with hot 1:1 HCl and rinsed with distilled water. The acid-washed glassware should be filled with distilled water and treated with all the reagents to remove any traces of phosphate that might adsorb on the glassware. Preferably, this glassware should be used only for the determination of phosphate and after use it should be washed and kept covered until needed again. If this is done, the treatment with 1:1 HCl and reagents is required only occasionally. Commercial detergents should never be used.

1.2 Colorimetric equipment: A spectrophotometer with an infrared phototube for use at 880 mμ, providing a light path of 1 inch or longer, should be used.

2. Reagents

2.1 Sulfuric acid solution, 8N: Dilute 112 ml of concentrated H_2SO_4 with distilled water to 500 ml.

2.2 Potassium antimonyl tartrate solution: Dissolve 4.3888 g $K(SbO)C_4H_4O_6.1/2\ H_2O$ in 200 ml of distilled water. Store in a dark bottle at 4°C.

---

*G. Edwards, A. Molof, and R. Schneeman, "Determination of Orthophosphate in Fresh and Saline Waters." Jour. AWWA, 57, 917 (1965).

2.3 Ammonium molybdate solution: Dissolve 20 g $(NH_4)_6Mo_7O_{24}\cdot 4\ H_2O$ in 500 ml of distilled water. Store in a plastic bottle at 4°C.

2.4 Ascorbic acid, 0.1M: Dissolve 1.76 g of ascorbic acid in 100 ml of distilled water. The solution is stable for about a week if stored at 4°C.

2.5 Combined reagent: Mix the above reagents in the following proportions for 100 ml of the combined reagent: 50 ml of 8N H_2SO_4, 5 ml of potassium antimonyl tartrate solution, 15 ml of ammonium molybdate solution, and 30 ml of ascorbic acid solution. Mix after addition of each reagent. All reagents must reach room temperature before they are mixed and they should be mixed in the order given. If turbidity forms in the combined reagent, shake and let it stand for a few minutes until the turbidity disappears before proceeding. The reagent is stable for one week if stored at 4°C.

2.6 Alcohol, ethyl (95 percent).

2.7 Stock phosphate solution: Dissolve in distilled water 0.2197 g of potassium dihydrogen phosphate, KH_2PO_4, which has been dried in an oven at 105°C. Dilute the solution to 1000 ml. 1 ml equals 0.05 mg P.

2.8 Working phosphate solution: Dilute 10.0 ml of stock phosphate solution to 1000 ml with distilled water. 1 ml equals 0.5 µg P.

2.9 Strong-acid solution: Slowly add 310 ml conc. H_2SO_4 to 600 ml distilled water. When cool, dilute to 1 liter.

3. Calibration

3.1 Standard curve: Prepare a series of standard phosphorus solutions to cover the range from 0 to 0.50 P mg/1. Make up the standards by diluting suitable volumes of working phosphate solution to 50 ml with distilled water.

3.2 Develop color in the series of standards and blank of distilled water as directed in 4. Procedure. Measure the color absorbance of each standard at 880 mμ with a spectrophotometer, using the reagent blank as the reference solution. Plot the absorbance values as ordinates and the corresponding phosphorus concentrations as abscissas.

3.3 Standards and blank should be processed exactly as the samples. At least one standard should be run either with each set of samples or once each day that analyses are performed. If the standard does not fall very close to the curve, then a new calibration curve should be prepared.

4. Procedure

4.1 Add 1 drop of phenolphthalein indicator to the 50.0 ml filtered sample. If a red color develops, add strong-acid solution dropwise to just discharge the color.

4.2 Add 2.5 ml of ethyl alcohol to sample and mix thoroughly.

4.3 Add 2.5 ml of combined reagent and mix thoroughly. After a minimum of 10 minutes, but no longer than 30 minutes, measure the color absorbance of each sample at 880 mμ with a spectrophotometer, using the reagent blank as the reference solution.

5. Calculation

5.1 Obtain concentration value of sample directly from prepared standard curve. Report results as soluble orthophosphate, mg P/l.

Soluble Orthophosphate

Method B*

Recommended by Dr. Donald Lear, Annapolis Field Station
Middle Atlantic Region, FWPCA

1. Apparatus

1.1 Glassware: A set of glassware cleaned in chromic acid cleaning solution must be used exclusive from general laboratory use. Periodic soaking of this glassware in cleaning solution, followed by thorough rinsing in phosphorus-free water, is recommended as routine procedure.

1.2 Colorimetric (same as Method A): A spectrophotometer with an infrared phototube for use at 880 mμ, providing a light path of 1 inch or longer, should be used.

2. Reagents

2.1 Sulfuric acid solution, 5N: Dilute 70 ml of concentrated H_2SO_4 with distilled water to 500 ml.

2.2 Potassium antimonyl tartrate solution: Weigh 1.3715 g $K(SbO)C_4H_4O_6.1/2\ H_2O$, dissolve in 400 ml distilled water in 500 ml volumetric flask, dilute to volume. Store in glass-stoppered bottle.

2.3 Ammonium molybdate solution (same as Method A): Dissolve 20 g $(NH_4)_6Mo_7O_{24}.4\ H_2O$ in 500 ml distilled water. Store in a plastic bottle at 4°C.

*J. Murphy and J. Riley, "A Modified Single Solution Method for the Determination of Phosphate in Natural Waters." Anal. Chim. Acta., 27, 31 (1962).

2.4 Ascorbic acid, 0.1M (same as Method A): Dissolve 1.76 g of ascorbic acid in 100 ml of distilled water. The solution is stable for about a week if stored at 4°C.

2.5 Combined reagent: Mix the above reagents in the following proportions for 100 ml of the mixed reagent: 50 ml of 5N H_2SO_4, 5 ml of potassium antimony tartrate solution, 15 ml of ammonium molybdate solution, and 30 ml of ascorbic acid solution. Mix after addition of each reagent. All reagents must reach room temperature before they are mixed and must be mixed in the order given. If turbidity forms in the combined reagent, shake and let it stand for a few minutes until the turbidity disappears before proceeding. The reagent is stable for one week if stored at 4°C.

2.6 Stock phosphate solution (same as Method A): Dissolve in distilled water 0.2197 g of potassium dihydrogen phosphate, KH_2PO_4, which has been dried in an oven at 105°C. Dilute the solution to 1000 ml. 1 ml equals 0.05 mg P.

2.7 Working phosphate solution (same as Method A): Dilute 10.0 ml of stock phosphate solution to 1000 ml with distilled water. 1 ml equals 0.5 µg P.

2.8 Strong-acid solution (same as Method A): Slowly add 310 ml concentrated H_2SO_4 to 600 ml distilled water. When cool, dilute to 1 liter.

3. Calibration (same as Method A)

3.1 Standard curve: Prepare a series of standard phosphorus solutions to cover the range from 0 to 0.50 P mg/l. Make up the standards by diluting suitable volumes of working phosphate solution to 50 ml with distilled water.

3.2 Develop color in the series of standards and blank of distilled water as directed in 4. Procedure. Measure the color absorbance of each standard at 880 mμ with a spectrophotometer, using the reagent blank as the reference solution. Plot the absorbance values as ordinates and the corresponding phosphorus concentrations as abscissas.

3.3 Standards and blank should be processed exactly as the samples. At least one standard should be run either with each set of samples or once each day that analyses are performed. If the standard does not fall very close to the curve, then a new calibration curve should be prepared.

4. Procedure

4.1 Add 1 drop of phenolphthalein indicator to the 50.0 ml filtered sample. If a red color develops, add strong-acid solution dropwise to just discharge the color.

4.2 Add 8.0 ml of combined reagent to sample and mix thoroughly. After a minimum of 10 minutes, but no longer than 30 minutes, measure the color absorbance of each sample at 880 mμ with a spectrophotometer, using the reagent blank as the reference solution.

5. Calculation (same as Method A)

5.1 Obtain concentration value of sample directly from prepared standard curve. Report results as soluble orthophosphate, mg P/l.

Soluble Orthophosphate

Method C*

Recommended by Mr. Paul Lefcourt, Hudson-Delaware Basins Office
Northeast Region, FWPCA

1. Apparatus (same as Method A)

1.1 Acid-washed glassware: All glassware used in the determination should be washed with hot 1:1 HCl and rinsed with distilled water. The acid-washed glassware should be filled with distilled water and treated with all the reagents to remove any traces of phosphate that might be adsorbed on the glassware. Preferably, this glassware should be used only for the determination of phosphate and after use it should be washed and kept covered until needed again. If this is done, the treatment with 1:1 HCl and reagents is required only occasionally. Commercial detergents should never be used.

1.2 Colorimetric equipment: A spectrophotometer with an infrared phototube for use at 880 mμ, providing a light path of 1 inch or longer, should be used.

2. Reagents

2.1 Sulfuric acid solution, 5N (same as Method B): Dilute 70 ml of concentrated H_2SO_4 with distilled water to 500 ml.

2.2 Potassium antimonyl tartrate solution: Dissolve 2.1944 g $K(SbO)C_4H_4O_6.1/2\ H_2O$ in 200 ml of distilled water. Store in a dark bottle at 4°C.

*G. Edwards, A. Molof, and R. Schneeman, "Determination of Orthophosphate in Fresh and Saline Waters." Jour. AWWA, 57, 917 (1965).

2.3 Ammonium molybdate solution (same as Method A): Dissolve 20 g $(NH_4)_6\ Mo_7O_{24}.4\ H_2O$ in 500 ml of distilled water. Store in a plastic bottle at 4°C.

2.4 Ascorbic acid, 0.1M (same as Method A): Dissolve 1.76 g of ascorbic acid in 100 ml of distilled water. The solution is stable for about a week if stored at 4°C.

2.5 Combined reagent (same as Method A): Mix the above reagents in the following proportions for 100 ml of the combined reagent: 50 ml of 8N H_2SO_4, 5 ml of potassium antimonyl tartrate solution, 15 ml of ammonium molybdate solution, and 30 ml of ascorbic acid solution. Mix after addition of each reagent. All reagents must reach room temperature before they are mixed and they should be mixed in the order given. If turbidity forms in the combined reagent, shake and let it stand for a few minutes until the turbidity disappears before proceeding. The reagent is stable for one week if stored at 4°C.

2.6 Acetone, reagent grade.

2.7 Stock phosphate solution (same as Method A): Dissolve in distilled water 0.2197 g of potassium dihydrogen phosphate, KH_2PO_4, which has been dried in an oven at 105°C. Dilute the solution to 1000 ml. 1 ml equals 0.05 mg P.

2.8 Working phosphate solution (same as Method A): Dilute 10.0 ml of stock phosphate solution to 1000 ml with distilled water. 1 ml equals 0.5 µg P.

2.9 Strong-acid solution (same as Method A): Slowly add 310 ml conc. H_2SO_4 to 600 ml distilled water. When cool, dilute to 1 liter.

3. Calibration (same as Method A)

3.1 Standard curve: Prepare a series of standard phosphorus solutions to cover the range from 0 to 0.50 P mg/l. Make up the standards by diluting suitable volumes of working phosphate solution to 50 ml with distilled water.

3.2 Develop color in the series of standards and blank of distilled water as directed in 4. Procedure. Measure the color absorbance of each standard at 880 mμ with a spectrophotometer, using the reagent blank as the reference solution. Plot the absorbance values as ordinates and the corresponding phosphorus concentrations as abscissas.

3.3 Standards and blank should be processed exactly as the samples. At least one standard should be run either with each set of samples or once each day that analyses are performed. If the standard does not fall very close to the curve, then a new calibration curve should be prepared.

4. Procedure

4.1 Add 1 drop of phenolphthalein indicator to the 25.0 ml filtered sample. If a red color develops, add strong-acid solution dropwise to just discharge the color.

4.2 Add 3.0 ml of acetone to sample and mix thoroughly.

4.3 Add 5.0 ml of combined reagent and mix thoroughly. After a minimum of 10 minutes, but no longer than 30 minutes, measure the color absorbance of each sample at 880 mμ with a spectrophotometer, using the reagent blank as the reference solution.

5. Calculation (same as Method A)

5.1 Obtain concentration value of sample directly from prepared standard curve. Report results as soluble orthophosphate, mg P/l.

APPENDIX 2

Tabulation of Phosphorus Recovery Values
by Laboratory, Using Methods A, B, & C.

Appendix 2

Tabulation of Phosphorus Recovery Values
by Laboratory, Using Methods A, B, & C.

Laboratory 1, Soluble Orthophosphate, mg P/1

	Method A	Method B	Method C
Sea Water	.281	.282	.282
+ Spike	.281	.278	.375
	.295	.280	.285
Av.	.286	.280	.314
Distilled Water	.230	.260	.275
+ Spike	.254	.230	.255
	.263	.236	.235
Av.	.249	.242	.255
Sea Water	.052	.057	.055
	.052	.051	.057
	.056	.052	.061
Av.	.053	.053	.058

Laboratory 2, Soluble Orthophosphate, mg P/1

	Method A	Method B	Method C
Sea Water	.356	.360	.335
+ Spike	.356	.360	.341
	.356	.360	.338
Av.	.356	.360	.338
Distilled Water	.225	.224	.230
+ Spike	.225	.223	.231
	.223	.224	.224
Av.	.224	.224	.228
Sea Water	.136	.132	.127
	.134	.132	.120
	.135	.135	.121
Av.	.135	.133	.123

Appendix 2 Contd.

Tabulation of Phosphorus Recovery Values by Laboratory, Using Methods A, B, & C.

Laboratory 3, Soluble Orthophosphate, mg P/1

	Method A	Method B	Method C
Waste Effluent	.588	.575	.535
+ Spike	.590	.580	.536
	.585	.570	.538
Av.	.588	.575	.536
Distilled Water	.245	.230	.215
+ Spike	.245	.230	.211
	.240	.228	.207
Av.	.243	.229	.211
Waste Effluent	.325	.340	.307
	.335	.340	.316
	.322	.348	.320
Av.	.327	.343	.314

Laboratory 4, Soluble Orthophosphate, mg P/1

	Method A	Method B	Method C
Polluted Fresh	.190	.110	.092
Water*	.190	.110	.092
+ Spike	.185	.110	.092
Av.	.188	.110	.092
Distilled Water	.225	.235	.220
+ Spike	.225	.235	.220
	.225	.235	.220
Av.	.225	.235	.220
Polluted Fresh	.005	.006	0
Water*	.005	.006	0
	.005	.006	0
Av.	.005	.006	0

*Contains 2.5 mg/1 of Fe.

Appendix 2 Contd.

Tabulation of Phosphorus Recovery Values
by Laboratory, Using Methods A, B, & C.

Laboratory 5, Soluble Orthophosphate, mg P/l

	Method A	Method B	Method C
Sea Water	.530	.505	.498
+ Spike	.530	.505	.498
	.530	.505	.498
Av.	.530	.505	.498
Distilled Water	.236	.230	.223
+ Spike	.240	.237	.227
	.239	.230	.225
Av.	.238	.232	.225
Sea Water	.290	.296	.285
	.291	.293	.287
	.290	.295	.287
Av.	.290	.295	.286
River Water	.287	.300	.272
+ Spike	.292	.287	.278
	.290	.292	.275
Av.	.290	.293	.275
Distilled Water	.236	.230	.223
+ Spike*	.240	.237	.227
	.239	.230	.225
Av.	.238	.232	.225
River Water	.060	.058	.066
	.054	.058	.056
	.051	.057	.054
Av.	.055	.058	.059

*Values repeated from above for comparison purposes.

Appendix 2 Contd.

Tabulation of Phosphorus Recovery Values
by Laboratory, Using Methods A, B, & C.

Laboratory 6, Soluble Orthophosphate, mg P/l

	Method A	Method B	Method C
River Water	.627	.614	.615
+ Spike	.603	.614	.598
	.603	.611	.615
Av.	.611	.613	.609
Distilled Water	.222	.221	.229
+ Spike	.218	.221	.227
	.219	.221	.233
Av.	.220	.221	.230
River Water	.375	.382	.379
	.375	.382	.379
	.381	.382	.379
Av.	.377	.382	.379

Appendix 2 Contd.

Tabulation of Phosphorus Recovery Values by Laboratory, Using Methods A, B, & C.

Laboratory 7, Soluble Orthophosphate, mg P/1

	Method A	Method B	Method C
Estuarine	.248	.249	.239
+ Spike	.251	.252	.244
	.253	.251	.247
Av.	.251	.251	.243
Distilled Water	.235	.237	.230
+ Spike	.239	.237	.236
	.237	.235	.261
Av.	.237	.236	.242
Estuarine	.016	.021	.022
	.022	.021	.021
	.025	.022	.021
Av.	.021	.021	.021
Fresh Water	.233	.235	.233
+ Spike	.234	.234	.231
	.236	.235	.232
Av.	.234	.235	.232
Distilled Water	.235	.237	.230
+ Spike	.239	.237	.236
	.237	.235	.261
Av.	.237	.236	.242
Fresh Water	.002	.002	.003
	.002	.002	.002
	.002	.003	.003
Av.	.002	.002	.003
Sea Water	.254	.258	.257
+ Spike	.258	.255	.254
	.259	.256	.258
Av.	.257	.256	.256
Distilled Water	.235	.237	.230
	.239	.237	.236
	.237	.235	.261
Av.	.237	.236	.242
Sea Water	.030	.030	.029
	.030	.030	.031
	.030	.030	.031
Av.	.030	.030	.030

*Values repeated from above for comparison only.

Appendix 2 Contd.

Tabulation of Phosphorus Recovery Values by Laboratory, Using Methods A, B, & C.

Laboratory 8, Soluble Orthophosphate, mg P/l

		Method A	Method B	Method C
	River Water	.226	.226	.233
	+ Spike	.227	.226	.230
		.227	.226	.230
	Av.	.227	.226	.231
Analyst 1	Distilled Water	.225	.225	.234
	+ Spike	.225	.225	.232
		.225	.225	.233
	Av.	.225	.225	.233
	River Water	.002	.001	.004
		.002	.001	.004
		.002	.001	.004
	Av.	.002	.001	.004
	River Water	.232	.232	.223
	+ Spike	.232	.233	.227
		.233	.233	.229
	Av.	.232	.233	.226
	Distilled Water	.232	.234	.237
Analyst 2	+ Spike	.232	.233	.237
		.233	.231	.233
	Av.	.232	.233	.236
	River Water	.008	.010	.013
		.009	.010	.012
		.010	.010	.012
	Av.	.009	.010	.012
	River Water	.230	.230	.224
	+ Spike	.231	.231	.227
		.231	.231	.228
	Av.	.231	.231	.226
	Distilled Water	.227	.230	.228
Analyst 3	+ Spike	.227	.230	.232
		.227	.230	.233
	Av.	.227	.230	.231
	River Water	.004	.006	.003
		.003	.005	.003
		.003	.005	.003
	Av.	.003	.005	.003